



Title	Some Experiments on the Food-Habit of Two Puzzling Species of Lady-Birds, <i>Epilachna pustulosa</i> Kôno and <i>E. vigintioctomaculata</i> Motschulsky (Coleoptera, Coccinellidae) (With 6 Text-figures)
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**Some Experiments on the Food-Habit of Two Puzzling  
Species of Lady-Birds, *Epilachna pustulosa* Kôno  
and *E. vigintioctomaculata* Motschulsky  
(Coleoptera, Coccinellidae)**

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(With 6 Text -figures)

The puzzling species, *Epilachna pustulosa* Kôno, has been at first described as a distinct species from her sister, *E. vigintioctomaculata* Motschulsky, a serious pest of potato in N. E. Asia, by the peculiarly expanded and tuberculated elytral edge and the preference of thistle as the natural food plant to potato (Kôno '37). Thereafter, it has been revealed by Watanabe & Sakagami ('48), who examined specimens from Hokkaido, that the elytral structure is considerably variable from population to population, but the legs of the former species are almost unexceptionally darker than those of the latter. Moreover, the feeding experiments proved that *E. pustulosa* could successfully grow with potato and other plants preferably attacked by *E. vigintioctomaculata*. Besides, there has been also discovered from various parts of Honshû. Using several populations of *E. pustulosa*, Yasutomi ('54) detected little difference of food-habit, succeeded in obtaining the fertile  $F_1$  between *pustulosa* and *vigintioctomaculata* and came to the conclusion that *these two species belong to one species*. However, it seems to the authors that his invaluable results indicate us yet only the starting point to analyse the problem but not the goal. If the fertile  $F_1$  was thus to be obtained from all the combinations *among the representative populations*, and, adopting the  $F_1$  fertility as *the criterium of conspecificity*, if all these populations were united into a single species, still we cannot deny the difference among populations existing in reality. Here we confront a problem of a species to be analysed both ecologically and genetically i.e., spatially and temporally. The present work deals with a bit of the comprehensive study in our laboratory and some feeding experiments were attempted on certain populations.

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### Material

An accurate diagnosis of the studied material is indispensable from its very nature of the present work mentioned above. The important characters and the data in collecting on the studied populations are shown in Tab. 1 and Fig. 1. Regret to say, we

Table 1. Main characters and data in collecting on three populations studied

abbreviation	Kp	Np	Sv
collected locality	Kamuikotan (Mt. Kamui)	Nopporo (Nopporo Virgin Forest)	Sapporo (Campus of Univ.)
collected date	VII 9. '53 (eggs & hibern. adults)	VI 12. '53-VIII 3. '53 (eggs & hibern. adults)	VII 17. '53 (eggs & hibern. adults)
collected from	thistle ( <i>Cirsium boreale</i> )	thistle ( <i>Cirsium boreale</i> )	potato ( <i>Solanum tuberosum</i> )
structure of elytral edge	distinctly expanded with distinct tubercle.	slightly expanded without tubercle	not expanded without tubercle
contour of elytra viewed laterally	normal, not declivous	normal, not declivous	normal, not declivous
*depression at elytral apex	absent	absent	present
coloration of venter	black, sometimes slightly with brownish tinge	black, sometimes slightly with brownish tinge	with dark brown tinge, some- times mostly black
coloration of leg	black	black	dark brown with black tinge
red-brown tinge of elytra	deep	deep	mild
central spots of elytra	mainly large, esp. first spot large & invert-heart shape	mainly large, esp. first spot large & invert-heart shape	mainly not so large as in Np & Kp, first spot without latero- posterior extension
taxonomical name	<i>pustulosa</i> (type-form)	<i>pustulosa</i> (non type-form)	<i>viginthioctomaculata</i>

\* After Dr. C. Watanabe

could not obtain the other type of *E. pustulosa* with declivous lateral elytral contour, without tubercle, remarkably expanded elytral edge, elytral depression and with blackish venter and legs. This type was at first discovered at Sōunkyō near Mt. Daisetsu in Hokkaido and thereafter the allied type was found also in Honshū. Other known types of *pustulosa-vigintioctomaculata* complex inhabiting Hokkaido would be perhaps represented morphologically by the tabulated three populations. Naturally the occurrence of other unknown populations possessing new characters or new combinations of characters would be possible.

The rearing of insects was carried out with Petri-schale of about 10 cm in diameter. Other procedures will be described in each experiment.



Fig. 1. Representative specimens of three populations studied. A : Sv (*E. vigintioctomaculata* of Sapporo). B : Np (*E. pustulosa*, non type-form of Nopporo). C : Kp (*E. pustulosa*, type-form of Kamuikotan)

### Experimental results

#### 1. Difference of larval mortality among three populations by the substitution of food-plant

To analyse the sensory cues which may play the leading rôle in food preference, the food-plant of some larvae was changed, corresponding to the development, from thistle to potato or invertly. The newly hatched larvae were chosen from each population sample and one part was reared with potato (P), another with thistle (T). By the daily examination on each schale containing at most 10 larvae, separation and transference of newly moulted larvae into other schales, or sometimes substitution of the food-plant were taken place. Thus certain individuals were reared, throughout the larval stage till the end of 4th instar, only with one sort of food-plant, namely PPPP or TTTT. On the other hand, the food plant of certain larvae was exchanged once at a definite moulting as such orders TPPP, TTPP etc. The mortality due to the substitution showed an interest difference among three populations as summarized in Table 2.

In the case of Np (cf. Table 1), the substitution caused in no lots the complete extinction of larvae. Always a certain number of individuals could survive

Table 2. Mortality difference of larvae among three populations, resulted by the substitution of food-plant

food-plant in each instar	Kp			Np			Sv		
	No. survived until substitution	No. emerged	type of survival	No. survived until substitution	No. emerged	type of survival	No. survived until substitution	No. emerged	type of survival
*TTTT							10	0	0
P*TTT							14	0	+ 0
PP*TT							24	0	++ 0
PPP*T							14	0	+++ 0
*PPPP	> 30	3	++++	20	5	++++	15	3	+++++
T*PPP	28	0	+ 0	15	2	++++	86	0	0
TT*PP	26	0	++ 0	5	2	++++			
TTT*P	8	0	+++ 0	3	1	++++			
TTTT*T	7	6	++++	5	3	++++			

P & T: Potato and thistle respectively. Substitution was marked with \*.  
 In the type of survival, 4 instars were represented with + (possible), or 0 (impossible).

until pupation and emergence. The substitution of P by T was not carried out. But, judging from the result mentioned above, it would be highly probable that also in such cases a similar result would have been obtained. In another word, the substitution of food-plant seems to give no distinct effect upon the mortality of larvae in Np. The mortality of Kp-larvae behaved somewhat differently. All of more than 30 individuals (exact number unrecorded, but about 40), reared immediately after hatching out died except 3 individuals, which could complete their development with potato until emergence. Moreover, in all cases of substitution T by P, all the individuals which could survive with thistle died sooner or later, after the transference into the potato diet was taken place. Therefore, we can assume a significant effect of substitution T by P on the mortality of Kp-larvae. Finally, Sv behaved completely in the opposite direction of Kp with respect to the substitution. All the larvae reared with thistle, after hatching out or after feeding with P during initial instars extincted without exception. The effect of substitution is, therefore, distinctly inverse in the case of Kp.

From these results it would be conceivable that Np can grow sufficiently with both food-plants. Kp and Sv, however, only with thistle or potato respectively. Or, the mortality of Kp and Sv is arranged on the opposite side of Np concerning with two sorts of food-plant. But, it would not be forgotten that at least 3 individuals of Kp could complete their development with potato. This fact suggests us the quantitative difference of platability rather than the qualitative one among different populations, namely the difference of % individuals which can grow with the opposite food-plant. This experiment being planned for another

purpose, its result is neither sufficient nor exact. The further analysis of the food habit was performed therefore in the following experiment.

2. *Difference of food refusal among three populations by the daily alternation of food-plant*

The principle of the experiment is very simple and consists of only the daily alternation of food-plant, namely, P-T-P-T . . . . . Thus the alternation of food-plant per 24 or occasionally 48 hours was succeeded until the pupation or death of the last remained individual in each schale which had contained about 10 individuals at the beginning of experiment. The amount of food consumption was daily measured by mm<sup>2</sup> and divided by individual number. Then the amount of food consumption pro individual of two successive days was summed up and the % ratio of potato consumption to each summed amount was calculated by  $(100 \times P)/(P+T)$  (P, T: Consumed amount of potato or thistle during one of two combined days). The same method was applied also to adults, but individual number pro schale was 4-5, or sometimes only 1-2, especially in cases of 1. generation of Np emerged in the laboratory. The experiment was succeeded under room temperature from VII-mid. until the death of the last individual for the hibernated adults and from VII-mid. to VII 21. for 1. generation adults emerged in the laboratory.

As seen in Table 3, the results of the experiment revealed a clear difference of food preference among three populations. In the case of Kp larvae consumed relatively large amount of potato in 2. instar, but still only 8.6 % and the mean potato consumption is only 3.2 %. The potato consumption of Sv-larvae was maintained throughout the larval stage above 99.3 % and the mean also 99.7 %. On the other hand, Np-larvae consumed about 40-50 % except 4. instar in which the value decreased until 28.4 %, partly caused by an occasional accumulation of complete refusal for potato in certain larvae which died on the same or next day. The mean % consumption shows 40.3 % and clearly differs from both Kp and Sv. The values of % potato consumption show therefore an ascending order of Kp, Np and Sv.

The potato consumption of adults took place in a very similar manner. In Kp both the hibernated and newly emerged adults expressed a complete denial for potato. But the 3 individuals which could grow with potato in the preceding experiment took nearly indifferently both food-plants by about 50 % potato consumption. Consequently, we can divide Kp into two distinct groups with respect to the disposition for potato, and if these two groups are considered together, still the mean value does not reach 15 %. The potato consumption of Np-adults is generally similar to that of larvae. In most of cases consumed potato was ca 40-60 %, irrespective of the difference of generations and food-plants previously taken. It is very curious that the group of 1. gen. alone, which was reared with potato, refused relatively much more potato (30.0 %). As to the disposition of Sv-adults, it would be almost needless to refer. The mean % consumption was identical with that of larvae, namely 99.7 %.

Judging from these results, the assumption based upon the preceding ex-

Table 3. Refusal difference for potato and thistle among three populations, resulted by daily alternation of food-plant

larvae								
instar	Kp		Np		Sv			
	mean % potato consumption	size of sample	mean % potato consumption	size of sample	mean % potato consumption	size of sample		
I	1.4%	4	40.0%	4	99.8	12		
II	8.6	10	42.5	8	100.0	4		
III	1.8	9	50.4	9	99.8	8		
IV	0.9	11	28.4	6	99.2	12		
mean	3.2	34	40.3	27	99.7	36		
adults								
gen. & food	Kp		Np		Sv			size of sample
	mean % potato consumption	size of sample	gen. & food	mean % potato consumption	size of sample	gen. & food	mean % potato consumption	
hibern. adult T (field)	0.0%	23	hibern. adult T (field)	42.9%	25	hibern. adult P (field)	100.0%	25
1. gen. TTTT (lab.)	0.2	8	1. gen. TTTT (lab.)	52.8	8	1. gen. PPPP (lab.)	99.3	12
1. gen. PPPP (lab.)	49.9	10	TPPP (lab.)	56.8	4			
			TTPP (lab.)	59.4	4			
			TTTP (lab.)	60.2	3			
			PPPP (lab.)	30.0	12			
			T(field) <sup>1)</sup>	62.6	10			
mean	12.2	41		47.4	76		99.8	37
	0.05*	31						

Gen. & food in adult: generation and food-plant during larval stage.

\* excluding the data on PPPP-individuals

1) 1. gen. adult collected from the potato field after the artificial transplantation.

periment would be firmly strengthened. Namely, Kp and Sv seem to stand

almost diametrically on the opposite side of Np with respect to the refusal for potato and thistle. From the behaviour of 3 Kp adults which could grow on potato and consumed it about as equal as thistle, it is surmised that the difference is probably dependent on a quantitative but not qualitative nature in the population constitution.

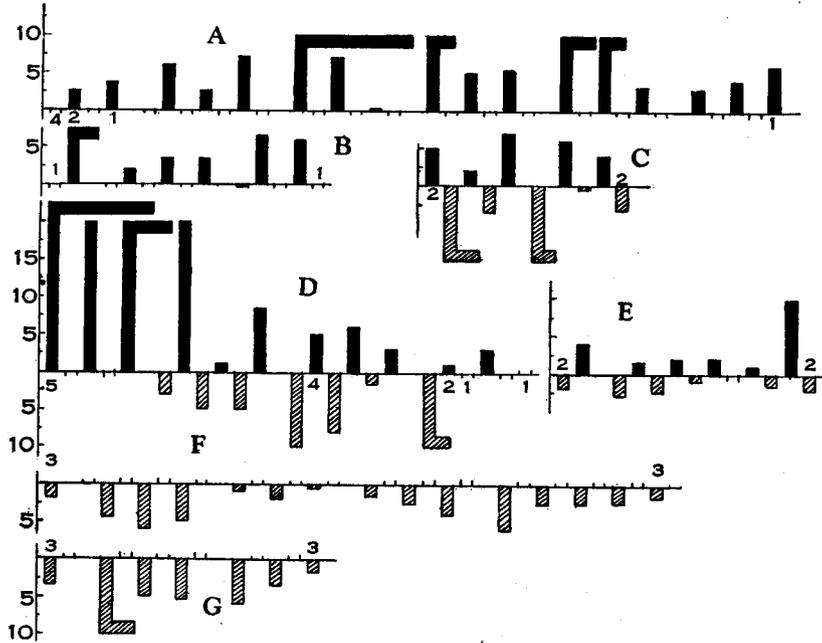


Fig. 2. Several examples on the alternation experiment. Abscissae: Days and ind. numb. tested; ordinate: Fed amount ( $\text{cm}^2$ ) of thistle (upper-black) and (lower-lined obliquely). A: Kp-hibernated adult. B: Kp-1.gen.. C: Kp-1.gen. (individuals which could complete the growth with potato). D: Np-hibern. adult. E: Np-1.gen.. F: Sv-hibern. adult. G: Sv-1.gen.)

From the results taken in adults, several examples were in Fig. 2 illustrated graphically. In addition to the distinct difference of three populations, an interest feature on the food consumption must be pointed out: Some adults of Np fed on potato but only after the initial refusal for a few days, as if certain duration would be necessary for adaptation. (Fig. 2, D).

### 3. Artificial transplantation of Np-larvae into the potato field.

In the preceding experiments, a wide adaptability of Np was proved in both larvae and adults. They feeds on both thistle and potato almost with no

preference, and be able to complete their development with potato at least under the laboratory condition. If this was clarified in the laboratory, how behaves Np to potato under the natural situation? The present experiment affords a part of answer for this question.

Through the kindness of Mr. H. Takahashi, we can perform an experiment with his potato field where is quite lady-bird-free. As diagrammed in Fig. 3, the

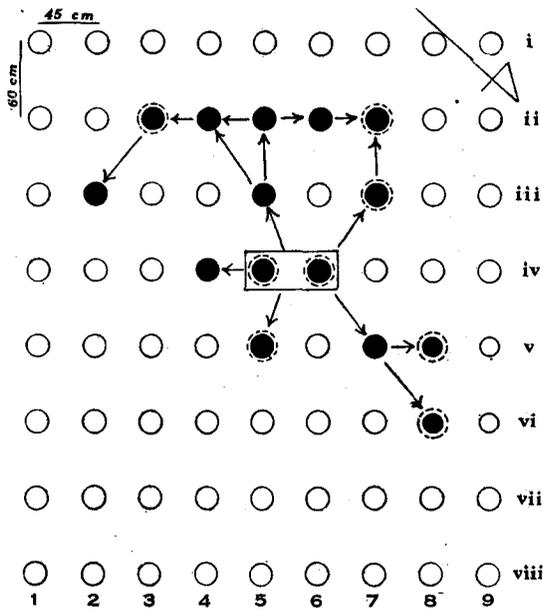


Fig. 3. Schematic representation of artificial transplantation of Np-larvae into the potato field and resulted migration.

field consists of 8 ridges (i-viii) running SE-NW and each consisting of 9 stocks (1-9). The transplantation was taken place on VII 5. '53 with 51 larvae of 1.-2. instar and ca 20 eggs. These were all attached on the leaves of stocks iv-5 and iv-6, planted about the centre of the field. The larvae were only occasionally rediscovered until VII 23. But a gradual expansion of the fed-spot peculiar to *Epilachna* was observed from stock to stock as shown in Fig. 3 with black circles. Several stocks as iv-4, iv-5 and ii-3 were almost perfectly damaged before cropping. During VIII 4.-13., 16 adults and 18 pupae or prepupae were collected from stocks shown in the figure with dotted ring. On VIII 31., namely after the cropping-

ing, 14 adults were collected on the leaves of cucumber stocks which had been planted in NE of the potato field and more or less attacked by these Np adults. Thus in total 48 individuals of 1. generation, or about 68 % of transplanted larvae and eggs could complete their development in this potato field. Considering the high mortality of insects during the developmental stages and the possibility that some individuals could complete their development but migrated into other places before collecting, this value of 68 % appears to be quite sufficient to prove the ability of Np to complete their development on potato under the natural condition. These collected Np individuals were reared thereafter in the laboratory, and certain of them were offered for the alternative

feeding experiment. Compared with other groups of Np, no clear difference in % potato consumption was found as seen in Tab. 3 (Np- 1. gen., T-(field)).

#### 4. Artificial transplantation of Np-adults into the egg-plant field

The transplantation experiment of Np-adults into the potato field failed owing to weather conditions. A similar transplantation was again tried with adults. In that time, the potato fields had been already cropped out in Sapporo. We had to use a field of egg-plant *Solanum M-longea*, located in the campus of our University. The field consists of first 5, then with addition of 2, in total 7 long riges running W-E. This field was heavily infested by *E. vigintioctomaculata* which had entered into the polyphagous stage and, because of the cropping out of potato, had migrated into this egg-plant field.

The western half of the field consisting of 5 ridges  $\times$  13 stocks was chosen as the experimental area. On IX 13., as a preliminary survey the number of *E. vigintioctomaculata* on each of 5 (i-v)  $\times$  13 stocks was carefully counted without handling the insect body. Individuals which fell down on the earth were again attached on a leaflet of the same stock. On the next day, IX 14., 7  $\delta$   $\delta$  and 10  $\varphi$   $\varphi$  of 1. generation Kp-adults were marked both sexually and individually with coloured paint and transplanted at random on stocks ii-iv  $\times$  4-10. As the control, 20  $\delta$   $\delta$  and 20  $\varphi$   $\varphi$  of *E. vigintioctomaculata* were collected from the other field, 10 of each were marked sexually and individually, other two 10-s only sexually and transplanted with the same manner. On the next day, IX 15., or the 1. day after the transplantation and thereafter the 2. and 4. day after the transplantation, all the stocks of i-v  $\times$  1-13 were carefully examined, the disappearance and interstock translocation were recorded. On X 23., or the 7. day after the transplantation, all the stocks in the experimental area and every cross-ridged 7 stocks with always 5 ones intermediate in each ridge in the other half of the field were examined and all the individuals, whether marked or not, were collected and recorded.

Table 4. Decrease of marked and transplanted Np- and Sv-adults in each successive examination of the exp. area in the egg-plant field

date of exam. after transplant.	no. of individuals remained in each successive examination						total of remained
	-1	0	1	2	4	7	
Np- $\delta$		7	6	5	3	3	3/7 42.9%
Np- $\varphi$		10	9	8	7	3	3/10 30.0
Np total		17	15	13	10	6	6/17 35.3
Sv- $\delta$		20	12	8	4	2	2/20 10.0
Sv- $\varphi$		20	12	10	6	5	5/20 25.0
Sv total		40	24	18	10	7	7/40 17.5
Sv non-marked	299					141	( $\delta$ 86+ $\varphi$ 65)

Signif. test betw. Np  $\delta$ -Np  $\varphi$ : (P=0.17)

Sv  $\delta$ -Sv  $\varphi$ : (P=0.17)

Np-Sv (marked): (P=0.087)

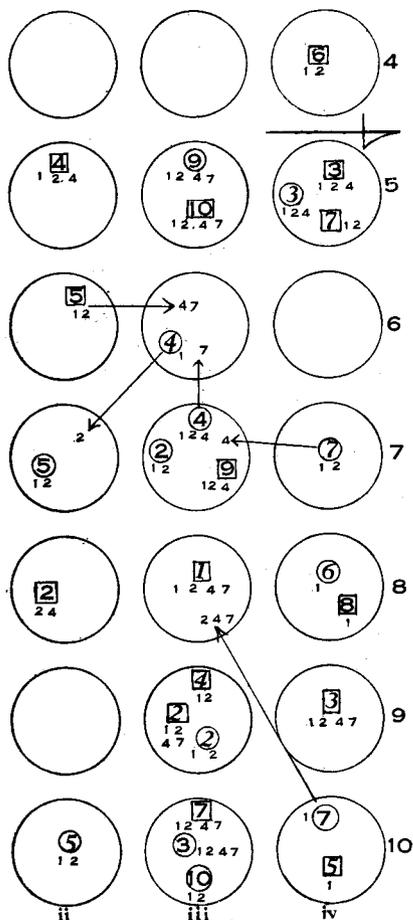


Fig. 4. Schematic representation of disappearance and inter-stock translocation of marked adults transplanted artificially into the egg-plant field. Number of stocks: ii-iv: ridge, 1-9: stock; Indiv. Number: Circle: Sv, Quadrat: Np, errected letter: ♀, italic: ♂, small letter added under the individual mark: days on which marked individuals were discovered on successive examination (1. 2. 4. and 7. day after transplantation).

Table 4 shows the decrease of marked individuals in each successive examination. The remained individuals of Np were, unexpectedly, much more in percentage (35.3 %) compared with Sv (17.5 %). The statistical test revealed, however, no significant difference in the rate of disappearance from the experimental area between Np ♂-♀ and Np-Sv, with 5 % level of significance, as far as the size of sample is limited in the present scale. Basing upon this result, it would be conceivable that the artificially transplanted Np-adults show no remarkable refusal for egg-plant under the natural condition.

As seen in Fig. 5, the adults of both Np and Sv seem to reside on one and the same stock during a considerable length of time. The translocation into the neighbouring stock was not so frequently observed. The locomotion is performed in the case of adults naturally by legs, but more frequently by flight sometimes to a quite distant place. Therefore, disappeared individuals seem to migrate into other fields. The migration into the rest half of the concerned field would be surely very probable. But no marked individuals could be found on the sampled stocks of the rest half on the examination taken on IX 23. In fact, in the preliminary survey on IX 13., the individual number of *E. vigintioctomaculata* found in this field was extraordinary high, their spatial distribution was very uneven and a gradient running W to E was observed to be formed. This overpopulated state, caused probably by the cropping out of the potato field situated W of

this egg-plant field, was thereafter depressed as seen in Figs. 5 & 6. Many individuals might migrate into the rest half of the field which was not examined on IX 23., and furthermore into other fields, f. ex. a large squash field situated E of the egg-plant field here considered. This migration of *E. vigintioctomaculata* after the cropping out of potato is surely a very interesting phenomenon and it would be probable that a certain number of disappeared marked individuals participate in this supposed migration. Finally, it must

be noted that many distinct fed-spots of the transplanted *Np*-adults were observed on the leaves of egg-plant on examination.

### Discussion

In the present study it has been confirmed that the food-habit of *pustulosa-vigintioctomaculata* differs not only between *E. pustulosa* and *E. vigintioctomaculata*, but also between two populations of the former species. Concerning the problem of conspecificity between these two puzzling species, the results give no sufficient evidences both positively or negatively. It is suggested, however, that various populations of *E. pustulosa* might differ each other not only in external characters, but also in food-habit. There seems to be probable that some morphological differences exist also among various populations of *E. vigintioctomaculata*, especially between populations of Honshû and Hokkaido as suggested partially by Watanabe (person, comm. to

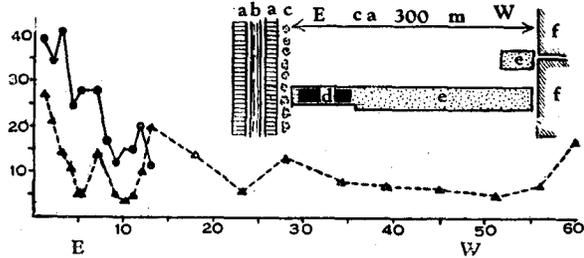


Fig. 5. Schematic representation of experimented situation and spatial distribution of adults *vigintioctomaculata* in each crossridge row on 13. IX '53 (---●---) and 23. IX '53 (—▲—▲—) in the egg-plant field. a: previous potato fields, b: stream, c: a row of trees, d: experimental area, e: egg-plant fields, f: squash fields; abscissae: crossridge row consisting of 5 (from 1.-13.) or 7 (14.-60.) stocks, numbered from E to W, ordinate: individual number in each crossridge row.

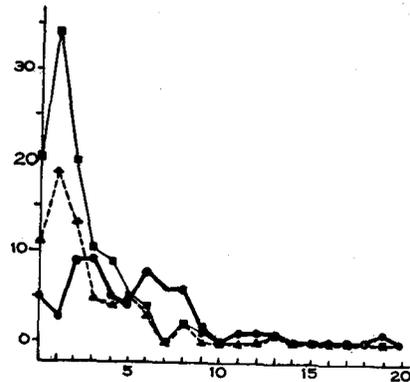


Fig. 6. Frequency of adult *vigintioctomaculata* in each stock. Ordinate: Number of stock; Abscissae: Individual number pro stock on 13. X '53 (---●--- in eastern area experimented) and on 23. IX '53 (▲—▲—▲— in eastern area experimented and —■—■— in the total field including sampling of western area not experimented).

the authors), Maki '51 and Ehara '53. It may be desirable that the careful comparison among various populations of *pustulosa-vigintioctomaculata*, instead of between these two species, are executed before (and after) concluding on the conspecificity between *E. pustulosa* and *E. vigintioctomaculata*. Finally, we would like to refer to their ecological distribution occurring in Hokkaido. The habitat segregation between Kp- and Sv-type populations may be clarified by their food preference, namely by the physiological connections to the habitat conditions (Mori '52: his second process in the habitat segregation). Another explanation must be made for Np-type populations. This type can feed on both potato and thistle, nevertheless it can be discovered only on the thistle except on the potato fields adjacent to the wild thistle communities (Kurosawa '53 and authors' obs.). At present we cannot show distinctly the factors which limit the habitat-range of Np-type populations below their physiological potential, in spite of their ability to complete the development with potato. Various factors may participate to the presentation of such a distribution pattern. But it seems to be probable that here the stability of the habitat plays an important rôle. The thistle provides continuously from spring until hibernation the suitable food and shelter for *E. pustulosa* in contrast with the potato, which is usually cropped out at the mid-summer. Moreover, the population density of *E. pustulosa* at their habitat hitherto recorded is seemingly not so enough high to necessitate the migration into other places. Therefore, the mere conservatism to the isolated suitable habitats may act considerably, if not completely, as an inhibiting factor for the dispersion of adults into the potato fields.

### Summary

1. The food-preference of two puzzling species, *Epilachna vigintioctomaculata*, a serious potato pest in Far East, and *E. pustulosa*, a pest of wild thistle, was investigated by the exchange of food-plant. Two populations of *E. pustulosa* (Kp: Type-form in Kamuikotan and Np: Non type-form in Nopporo) and one population of *E. vigintioctomaculata* (Sv: Sapporo) were studied as the representative populations.

2. The mortality after exchange of the food-plant differs among three populations. Kp and Sv could not complete the development with potato or thistle respectively, but Np and a few of Kp could complete with both plants. This difference was further confirmed by the daily alternation of food-plant.

3. The transplantation of Np-larvae into the potato field revealed the successful growth of a considerable rate of Np with potato under the natural conditions.

4. The transplantation of Np-adults into the egg-plant field showed no marked difference between Np and Sv as to the rate of disappearance from the field.

5. All these results seem to suggest the existence of difference in the food-habit not only between *E. pustulosa* and *E. vigintioctomaculata* but also between two types of *E. pustulosa*.

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